PB85-146850

Toxicity of Trivalent Chromium to Early Life Stages of Steelhead Trout

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1984

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1. REPORT NO.	2.	3.
EPA-600/J-84-178		PB85-146850

4. TITLE AND SUBTITLE	5. REPORT DATE
Toxicity of Trivalent Chromium to Early Life Stages of Steelhead Trout (Journal Version)	1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Donald G. Stevens and Gary A. Chapman	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory Office of Research and Development 200 SW 35th Street Corvallis, Oregon 97331	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Same	Journal Article
	14. SPONSORING AGENCY CODE
	EPA-600-02

15. SUPPLEMENTARY NOTES

Environmental Toxicology and Chemistry, Vol. 3, pp. 125-133, 1984

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	10
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

EPA-600/J-84-178
JOURNAL ARTICLE

Environmental Toxicology and Chemistry, Vol. 3, pp. 125-133, 1984
Printed in the USA. Pergamon Press Ltd.

0730-7268/84 $3.00 + .00
1984 SETAC

TOXICITY OF TRIVALENT CHROMIUM TO EARLY LIFE STAGES OF STEELHEAD TROUT

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(*Received* 28 *June* 1983; *Accepted* 27 *October* 1983)

Abstract — Acute and early life stage toxicity tests were conducted with trivalent chromium and steelhead trout (*Salmo gairdneri*). A 96-h LC_{50} of 4,400 μg/L chromium was obtained with two-month-old juvenile fish. Early life stage exposure from newly fertilized eggs to 30-d post-swimup produced complete mortality at 495 μg/L, and significantly reduced survival to hatch and to the end of the test at 157 and 89 μg/L, respectively. An early life stage test started with eyed eggs produced similar results, with hatching survival significantly reduced at 271 μg/L. An acute test conducted with fish surviving the early life stage test showed that no acclimation resulted from previous chromium exposure.

Keywords — Chromium Steelhead trout Acute toxicity Chronic toxicity Early life stage

INTRODUCTION

Chromium salts are used in many industrial processes, including electroplating, tanning and textile manufacturing. Other sources include cooling towers, paints, fungicides and wood preservatives. Chromium occurs in several valence states, of which the trivalent and hexavalent forms are biologically and environmentally significant, although they differ in chemical characteristics [1]. Trivalent chromium (e.g., the chromium nitrate used in this study) will form complexes with negatively charged organic and inorganic species, and its solubility and toxicity vary with water quality characteristics.

Published toxicity data for trivalent chromium include several acute toxicity studies with rainbow trout, but no early life stage or other chronic tests have been reported for salmonids. Pickering and Henderson [2] demonstrated the effects of hardness on the toxicity of trivalent chromium to fathead minnows (*Pimephales promelas*) in static tests. They obtained 96-h LC_{50} values for fathead minnows of 5,070 and 67,400 μg/L at hardnesses of 20 and 360 mg/L, respectively. Bills et al. [3] obtained a nominal 96-h LC_{50} value of 11,200 μg/L in static tests with rainbow trout. Hale [1] conducted flow-through tests and obtained a measured 96-h LC_{50} value of 24,000 μg/L with rainbow trout. However, water hardness was not reported in either of these studies.

This study was conducted to determine the acute and chronic effects of trivalent chromium, added as chromium nitrate, on the early life stages of steelhead trout (*Salmo gairdneri*). Two 96-h acute and two chronic early life stage toxicity tests were conducted. Chromium exposure during the first chronic test lasted only 30

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d, beginning with newly fertilized eggs. Other eggs were concurrently exposed to nitric acid to serve as pH controls for the higher levels of chromium. Exposure during the second chronic test lasted 72 d for newly fertilized eggs and 58 d for the exposure started at the eyed stage. These exposures were concurrent and continued to 30 d post-swimup.

One acute toxicity test was conducted to determine if continuous exposure to chromium induced acclimation among juveniles surviving the early life stage test. The other acute toxicity test provided a standard 96-h LC_{50} for extra control juveniles from the population of eggs used as the source of embryos for the early life stage test.

METHODS AND MATERIALS

Two proportional diluters [4], each delivering five concentrations and a control, were used to provide up to 10 chromium concentrations and two controls. The tests were conducted in 19-liter aquaria with a drain height to maintain a 14-liter volume. The 50% replacement time of test water in the aquaria was approximately 25 min. Black plastic was used to shroud the aquaria against outside disturbance and light. Daytime illumination was provided by two 40-w fluorescent tubes controlled by a timer and adjusted weekly to coincide with the natural photoperiod. Water temperature was controlled and was recorded continuously in one aquarium.

Chromium nitrate [$Cr(NO_3)_3 \cdot 9H_2O$] was the chromium source in all tests. Concentrations under 500 μg/L were analyzed on a Perkin-Elmer 305b atomic absorption spectrophotometer equipped with a carbon tube atomizer. Concentrations of 500 μg/L and above were analyzed using a nitrous oxide flame on a Perkin-Elmer 403 atomic absorption spectrophotometer. Samples for chromium analysis were taken weekly from each concentration, and daily samples were taken from selected concentrations to monitor diluter operation. Water samples for chromium analysis were split and one portion filtered through 0.45-μm Millipore filters for estimation of dissolved chromium. The rapid test solution replacement rates of the test chambers were assumed to be high enough to avoid significant conversion of trivalent chromium to hexavalent chromium.

Dissolved oxygen was maintained near saturation. Hardness was monitored daily and controlled by treatment of incoming water with a reverse osmosis unit and adjusted by mixing with the ambient water. Routine water analyses were performed using standard methods [5].

Eggs for these tests were acquired from the Oregon Department of Fish and Wildlife North Fork (Alsea River) hatchery. The eggs were fertilized at the hatchery and water-hardened 1 h before transport to the laboratory. Test organisms for the acute test and the eyed-egg portion of the second chronic test were from the same group of eggs reared under control conditions prior to tests.

Early life stage test

The first early life stage test required two diluters to provide eight concentrations of chromium and two controls. A third identical diluter was equipped to deliver five concentrations of nitric acid and a control. The highest acid concentration was intended to have the same pH as the 25,000 μg/L chromium concentration. The pH of this concentration was 5.2 in static tests conducted to determine the range of concentrations for the continuous-flow tests. Nominal concentrations of trivalent chromium ranged from 277 to 25,000 μg/L. Each aquarium contained an incubator consisting of a polyvinylchloride cylinder 10.5 cm in diameter and 23 cm high, with a glass bottom and removable wood lid (Fig. 1). Water entering the aquarium flowed upward through the incubator and into the aquarium through four screened holes near the top of the cylinder. The incubator was half-filled with three

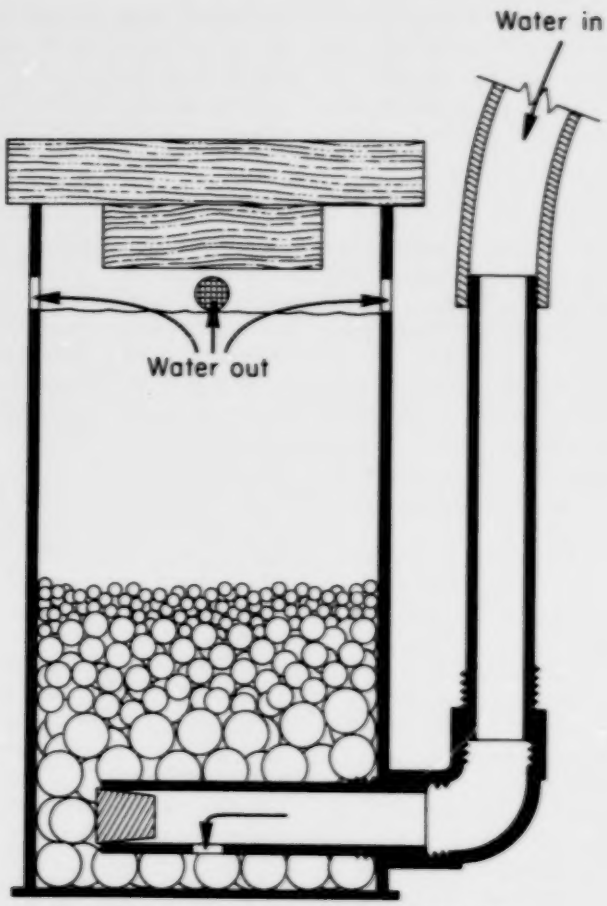

Fig. 1. Diagram of incubator used for early life stage tests.

sizes of marbles — 1.4, 1.0 and 0.4 cm in diameter — the smaller being layered on the top. The marbles served as a flow diffuser and as an irregular substrate to minimize egg movement and provide resting spaces for larvae. The volume of the incubator was slightly over 1 liter and the 50% partial replacement time was 1.5 min [6]. Fertilized eggs were randomly assigned to the incubators using 35 ml of eggs to give approximately 150 eggs per concentration.

The fertilized eggs were put into the incubators 3 h after fertilization. The diluter was already up to concentration when the eggs were added. Unfertilized eggs or eggs containing dead embryos were removed daily and preserved in Stockards solution [7], which cleared the eggs. These eggs were examined later to determine the presence or absence of embryonic development. Unfertilized eggs were counted and the number subtracted from the totals at each concentration. This test was continued for 32 d, which allowed complete hatching in the controls, and was then terminated to begin a second early life stage test at lower chromium concentrations.

Two diluters were also used for the second chronic test to provide 10 concentrations of chromium and two controls. The nominal concentrations of trivalent chromium were lowered from the first test and

ranged from 6 to 500 μg/L. The same incubators were used and 25 ml of eggs was added to give approximately 150 eggs per concentration. On day 14 of the test, surplus eyed eggs were added to separate test incubators to determine the effects on embryos which had developed to the eyed stage prior to chromium exposure.

When the controls reached swimup, five fish from each control group were removed and weighed to determine feeding rates. All the fish were fed Oregon Moist Pellet starter mash three times a day at 4% of body weight per day. This ration was about 15% body weight per day when both ration and fish weights were converted to dry weights. Food was weighed out weekly and adjusted for mortality. The juvenile steelhead were fed for 30 d after swimup.

At the end of the feeding period, the fish were starved for 2 d and 20 fish were removed from each aquarium for blotted wet weight and standard length measurements. The 20 fish were collected by crowding all the fish to one end of the aquaria, where they could not easily avoid the dip net, to get a reasonably random sample. The weight and length data were analyzed using Dunnett's multiple comparison test [8]. The remainder of the surviving fish were held for use in an acute toxicity test to assess the development of acclimation. The chronic survival data were analyzed using the method of Elaudt-Johnson and Johnson [9], a *t* test to compare the controls and the chi-square test with correction for continuity [10] to determine significant differences between the controls and treatments.

Acute tests

Two acute toxicity tests were run. One was a 96-h test to measure whether acclimation occurred from previous chromium exposure, and the other was a standard 96-h LC_{50} test to establish an acute/chronic toxicity ratio. Both tests were conducted with the proportional diluters used for the chronic studies. Fish for the acclimation acute toxicity test were taken from the survivors of the early life stage test, including controls and fish from all concentrations from 13 to 157 μg/L.

Following the removal of some fish for weight and length measurements at the end of the early life stage chronic test, the remaining fish were fed for 1 d and starved for 1 d before transfer to the acute test aquaria. To separate the treatment groups, 90 fish from each chronic treatment concentration were apportioned among six cylindrical stainless steel screen cages, 23 cm high and 8 cm in diameter, each with a plastic petri dish for a bottom. Fifteen fish were placed in each cage (except at the chronic exposure level of 157 μg/L, in which the 30 surviving fish were distributed equally among three cages). When all the cages contained the proper number of fish, one cage from each chronic concentration was transferred to each acute concentration. Routine water analyses and observations for dead fish were conducted daily.

For the standard acute toxicity test, 40 fish (20 per duplicate aquaria) were placed in each concentration, and two diluters provided eight chromium concentrations from 1,100 to 5,825 μg/L and two controls. The fish were from the same stock used in the chronic test but were reared under control conditions. The mean weight and length of the fish was 0.69 g and 37 mm, respectively. A 96-h LC_{50} value was calculated using the Trimmed Spearman-Karber method [11].

RESULTS AND DISCUSSION

The first of two chronic tests was terminated after 32 d. The poor survival at the lowest concentration (277 μg/L) indicated that the chromium concentrations were too high and that the test needed to be repeated at lower concentrations. The means for water hardness and temperature for this test were 25.7 mg/L and 12.6°C, respectively. The data are presented here to indicate the results of the acid controls. The reduced survival in the high acid control (pH 5.67) (Table 1) indicated that pH alone could have

Table 1. Effect of chromium nitrate and pH on survival of steelhead trout embryos exposed from fertilization through hatching

Nominal chromium concentration (μg/L)	Mean $[H^+]$ (as pH)	Survival (%)
25,000	5.45	0
7,800	6.32	0
2,458	6.95	0
1,376	7.04	0
922	7.12	1
617	7.14	0
413	7.17	1
277	7.20	27
High acid control	5.67	77
Low acid control	6.35	97
Control[a]	7.20	94
Control	7.42	91
Control	7.33	93

[a]Controls are from each of three diluters used for tests.

produced considerable mortality at the highest chromium concentration of 25,000 μg/L (pH 5.45). However, the low acid control (pH 6.35) did not cause mortality, indicating that mortality at chromium concentrations of 7,800 μg/L and lower was not caused by the acidity of the chromium solutions.

Complete mortality in the second chronic test occurred at 495 μg/L, with nearly complete mortality at 271 μg/L chromium in the early life stage test started from fertilized eggs (Table 2). These results are similar to those obtained in the first chronic test. Embryo survival was significantly reduced ($p = 0.05$) at 157 μg/L. Significant total mortality, from fertilization to the end of the test, occurred at 89 μg/L. Statistical analysis using the Dunnett's test did not indicate any significant reduction in weight and length consistent with a dose response (Table 3). There was a gradual decrease in both weight and length in those fish from eggs exposed from fertilization. However, variability within treatments precluded consistent significant differences.

Chromium appeared to have the greatest effect during the embryonic stage, with most of the mortality occurring at hatching. Nearly all the embryos developed normally, but hatching appeared to be delayed about 24 h in all concentrations of chromium. Of those dying at hatch, 67% of the embryos were unable to break the chorion, while the rest died with only the head or part of the tail protruding through the chorion. Many that survived spent a day or more with the head and yolk sac still within the chorion, suggesting interference with the release or the efficacy of the choriolytic enzymes the embryos normally produce at hatching [12]. At

Table 2. Effect of chromium nitrate on survival of steelhead trout exposed from fertilized egg to feeding fry

Mean measured total chromium concentration (μg/L ± SD)	No. of eggs per concentration (without blanks)[a]	Survival				
		Fertilization to hatching		Hatching to end of test		Total
		n	%	n	%	%
Control (diluter 1)	132	128	97	118[b]	96	93
Control (diluter 2)	135	135	100	125[b]	96	96
<5	136	135	99	124	92	91
9 ± 1.7	146	143	98	131	92	90
13 ± 1.5	141	140	99	130	93	92
19 ± 1.2	146	142	97	136	96	93
30 ± 1.3	134	131	98	119	91	89
48 ± 2.7	136	133	98	121	91	89
89 ± 14.8	140	122	87	119	98	85[c]
157 ± 7.3	137	60	44[c]	53	88	39[c]
271 ± 22.2	145	4	3[c]	3	75	2[c]
495 ± 48.8	139	0	0[c]	—	—	—

[a]There were no more than two blanks in any group of eggs.
[b]Excludes five fish removed for weighing to determine ration size.
[c]Significantly different from controls ($p = 0.05$) using chi-square test.

Table 3. Effect of chromium nitrate on the final weight and length of steelhead trout in early life stage chronic exposures started with newly fertilized eggs or eyed eggs

Mean measured total chromium concentration (μg/L)	Mean fish weight (g)		Mean fish length (mm)	
	Fertilized eggs	Eyed eggs	Fertilized eggs	Eyed eggs
Control	0.346	0.302	31.0	29.4
<5	0.328		30.0	
9	0.330		30.2	
13	0.324		30.3	
19	0.382		30.1	
30	0.306		30.1	
48	0.299	0.314	28.8[a]	30.1
89	0.306	0.301	29.3[a]	30.3
157	0.316	0.295	29.9	29.7
271	0.280[b]	0.280	29.0[b]	29.3

[a]Significantly different from controls ($p = 0.05$) using Dunnett's Multiple Comparison Test.
[b]Value not significant because of small sample size.

the higher concentrations, the chorions persisted for 3 weeks following hatching, while they disappeared within a few days in the controls. The chorions from chromium-exposed eggs were rigid and resembled split tennis balls. Chromium, which can be antibacterial, may have inhibited the bacteria that normally complete the dissolution of the chorions.

Embryos exposed to chromium starting from the eyed stage showed a greater tolerance than those exposed from fertilization. The concentration causing significant mortality of eyed embryos was 271 μg/L, compared with 89 μg/L for those exposed from fertilization (Tables 2 and 4). This would be expected if the effect were a simple concentration–time relationship. Embryos in both experiments exhibited high mortality during hatching and both had persistent chorions at high chromium concentrations. The high mortality (24%) among these controls following hatch may have been disease related. These control fish did not feed actively and many that died had dark tails and were pin-headed, indicating that they probably never began to feed. No signs of disease were seen in other aquaria. Temperature records indicate that the temperature history was similar between both groups of eggs when exposure for the eyed stage began, ruling out the possibility that poor feeding was the result of earlier temperature stress or differences in rate of development.

Summaries of the water chemistry during the second chronic test and both acute tests are given in Tables 5 and 6. The percent of the chromium determined to be dissolved ranged from 93 to 94% of total in the 5 to 13 μg/L concentrations, from 83 to 89% in the 19 to 495 μg/L concentrations, and from 50 to 75% for concentrations above 1,100

Table 4. Effect of chromium nitrate on survival of steelhead trout exposed from eyed embryos to feeding fry

Mean measured total chromium concentration (μg/L)	Total no. of eggs per concentration	Survival				
		Eyed embryo to hatching		Hatching to end of test		Total
		n	%	n	%	%
Control	126	123	98	94	76	75
48	129	128	99	117	91	91
89	125	123	98	114	93	91
157	132	124	94	113	91	86
271	131	38	29[a]	33	87	25[a]
495	130	0	0[a]	0	0	0[a]

[a]Significantly different from the controls ($p = 0.05$) using chi-square test.

Table 5. Summary of water chemistry during second early life stage chronic test

Parameter	Mean	Range
Hardness (mg/L as $CaCO_3$)		
Control	25.0 (48)[a]	20–31
pH		
Control	7.33 (17)	7.05–7.53
495 μg/L	7.25 (11)	7.08–7.43
Alkalinity (mg/L as $CaCO_3$)		
Control	24.0 (16)	20–27
495 μg/L	21.5 (11)	19–23
Temperature	12.8 (147)[b]	11.9–14.5

[a]Number of measurements in parentheses.
[b]Mean temperature determined by averaging temperature recorded daily at 6 a.m. and 6 p.m. (approximate daily minimum and maximum).

μg/L. All chromium concentrations are reported as total chromium.

The acute test conducted with the surviving fish from the chronic test did not indicate that acclimation had occurred. In fact, fish chronically exposed to 48 and 89 μg/L of chromium were less tolerant than those exposed to lower concentrations (Table 7). Fish exposed to 157 μg/L from the eyed stage had less mortality than those exposed to lower levels, but the sample was small. Because the data on the whole do not show a trend toward acclimation, the apparent acclimation at 157 μg/L may have been incidental or the result of selection of tolerant larvae.

The data from the chronic tests indicate that significant reduction in growth (length or weight) would probably occur only at chromium concentrations also producing significant mortality. A slight but statistically significant decrease in survival occurred at 89 μg/L and very poor survival was seen at 157 μg/L.

The biological significance of the small reductions in percent survival and growth observed at 48 and 89 μg/L is uncertain. Since early life stage tests are generally accepted as surrogates for life-cycle chronic tests, slight abberations relative to control survival, development and growth take on greater significance as indicators of potential cumulative effects than they might otherwise warrant. In this respect, the early mortality at 89 μg/L may represent a less important biological effect than the statistically uncertain reductions in length and weight occurring at 45 and 89 μg/L. The chronic toxicity threshold for this test is bounded by the 30 μg/L concentration of no apparent effect and the 157 μg/L concentration of unacceptable toxic effect.

Table 6. Water chemistry determinations for acute tests

Mean measured total chromium concentration (μg/L)	Hardness (mg/L as $CaCO_3$)	pH	Alkalinity (mg/L as $CaCO_3$)	Dissolved oxygen (mg/L)	Mean temperature (°C)[a]
Acclimation test					
Control	25	7.53	23	10.0	—
2,125	—	7.11	21	—	12.9
3,200	—	6.95	20	—	—
4,625	—	6.72	17	10.3	—
7,900	21	6.43	14	—	—
12,200	15	6.11	6	—	—
Acute test					
Control	25	7.42	26	10.3	—
1,100	—	7.13	19	—	—
1,933	—	6.76	22	—	—
1,966	—	6.80	20	—	—
2,900	—	6.70	20	—	—
3,100	—	6.78	18	—	12.5
3,966	—	6.84	19	10.1	—
5,650	—	6.59	13	—	—
5,825	—	6.58	15	—	—

[a]Mean temperature determined by averaging temperature recorded daily at 6 a.m. and 6 p.m. (approximate daily minimum and maximum).

Table 7. Percent mortality of juvenile steelhead trout acutely exposed to chromium nitrate following chronic embryo-larval exposure to lower levels of chromium

Chronic exposure level (μg/L)[a]	Mean total chromium concentration for acute exposure (μg/L)					
	Control	2,125	3,200	3,625	7,900	12,200
From fertilized eggs						
Control	0	0	7	40	100	100
Control	0	0	0	27	100	100
13	0	0	0	40	100	100
19	0	0	0	33	100	100
30	0	0	0	33	100	100
48	0	0	0	53	100	100
89	0	7	7	73	100	100
From eyed eggs						
Control	7	7	7	33	100	100
48	0	7	0	60	100	100
89	0	0	0	33	100	100
157[b]	—	—	0	20	100	—

[a]15 fish per concentration, except where noted.
[b]Only enough survivors to allow 10 fish for three concentrations.

For steelhead reared under control conditions, the 96-h LC_{50} for chromium was determined to be 4,400 μg/L (95% confidence interval = 4,260, 4,612) (Table 8). This value is similar to those reported for fathead minnows and guppies for trivalent chromium (chromium potassium sulfate) in soft water [2]. In hard water the 96-h LC_{50} for fatheads was 67,400 μg/L, compared with 5,070 μg/L in soft water. The toxicity of chromium salts varies not only with hardness, but also varies widely with temperature, pH, valence of the chromium and the species tested [13]. However, under the conditions of these tests with steelhead trout, the acute/chronic ratio for trivalent chromium was between 0.007 and 0.036 using chronic values of 30 and 157 μg/L, respectively.

Table 8. Mortality of steelhead trout exposed to chromium nitrate for 96 h

Mean measured total chromium concentration (μg/L ± SD)	Percent mortality[a]		
	48 h	72 h	96 h
Control	0	0	0
1,100 ± 173	0	0	0
1,933 ± 252	0	0	3
1,966 ± 289	0	0	0
3,000 ± 283	0	0	0
3,100 ± 265	0	0	0
3,966 ± 379	5	10	20
5,650 ± 412	68	100	100
5,825 ± 699	100	100	100

[a]40 fish per concentration.

Acknowledgement — We wish to thank J.D. Andros for statistical analysis of the data, W.L. Griffis, J. Sanders, A. Sipols, J. Waters and C.W. Wise for the chromium analysis, and the Oregon Department of Fish and Wildlife for the steelhead eggs.

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